



## Agarose Gel Analysis of Plasmid DNA

The main uses of agarose gels for plasmid DNA analysis are:

- ▶ Analysis of the size and conformation of nucleic acids in a sample
- ▶ Quantification of DNA (see page 16)
- ▶ Separation and extraction of DNA fragments

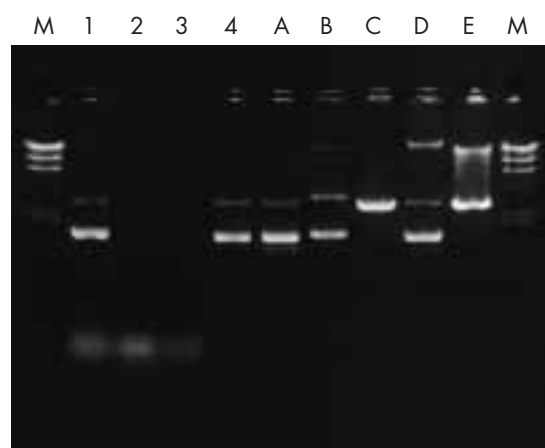
### Analysis of a purification procedure

**Figure 5** shows how agarose gel electrophoresis can be used to analyze the nucleic acid content of samples taken during a plasmid purification procedure. The gel demonstrates successful plasmid purification using anion-exchange columns as well as some atypical results.

**M:** Lambda DNA digested with *Hind*III.

- 1:** Cleared lysate containing supercoiled (lower band) and open circular plasmid DNA (upper band) and degraded RNA (smear at the bottom of the gel).
- 2:** Flow-through fraction containing only degraded RNA (the plasmid DNA is bound to the anion-exchange resin in the column).
- 3:** Wash fraction to ensure that the resin in the column is cleared of RNA and other contaminants (plasmid DNA remains bound to the column).
- 4:** Eluate containing pure plasmid DNA in supercoiled and open circular forms.

### Analysis of the Plasmid Purification Procedure



**Figure 5.** Agarose gel analysis of a plasmid purification procedure using QIAGEN anion-exchange tips. Samples were taken at different stages of the procedure. 2  $\mu$ l of each sample was run on a 1% agarose gel. **M:** lambda-HindIII markers.

Lanes A–E illustrate some atypical results that may be observed in some preparations, depending on plasmid type and host strain.

- A:** Supercoiled (lower band) and open circular form (upper band) of the high-copy plasmid, pUC18, with an additional band of denatured supercoiled DNA migrating just beyond the supercoiled form.
- B:** Multimeric forms of supercoiled plasmid DNA (pTZ19) that may be observed with some host strains and should not be mistaken for genomic DNA. Multimeric plasmid DNA is easily distinguished from genomic DNA by restriction digestion.
- C:** Linearized form of plasmid pTZ19 after restriction digestion with *Eco*RI.
- D:** Sample contaminated with bacterial chromosomal DNA (uppermost band).
- E:** *Eco*RI digestion of a sample contaminated with bacterial genomic DNA, which gives a smear above the plasmid DNA.

**Tip** With large-constructs such as BAC, PAC, and P1 DNA, the supercoiled form migrates at a slower rate than the linear form. Furthermore, large-construct DNA >50 kb is often difficult to distinguish from genomic DNA by agarose gel analysis.

### Gel extraction

Agarose gels can be used for separation and extraction of DNA fragments, for example, a specific DNA fragment from a PCR or restriction digestion reaction.

**Tip** Ensure that the percentage of agarose used for the gel allows good separation of DNA fragments for easy excision.

**Tip** Run agarose gels for DNA extraction at a low voltage. This will enable efficient separation of DNA bands without smearing, facilitating excision of the gel slice.

**Tip** Excise the fragment quickly under low-strength UV light to limit DNA damage.



DNA fragments can be extracted quickly and efficiently from agarose gels using silica-gel-based purification. Silica-gel-based methods typically result in higher and more reproducible recoveries than other gel extraction methods, such as electroelution, and require no phenol extraction or ethanol precipitation. In a typical silica-gel-based purification procedure, the agarose gel slice is first solubilized. DNA is then bound to the silica-gel material in the presence of high concentrations of chaotropic salts. A wash step removes impurities, and DNA is then eluted in low-salt buffer.

**Tip** QIAGEN offers three kits for silica-gel-based purification of differently sized DNA fragments from agarose gels, which differ in methodology and elution volumes:

- ▶ Purification of fragments between 70 bp and 10 kb in a spin-column format which can be used in a microcentrifuge or on a vacuum manifold.
- ▶ Purification of fragments between 70 bp and 4 kb in a spin-column format, in elution volumes of only 10  $\mu$ l.
- ▶ Purification of fragments between 40 bp and 50 kb using silica-gel particles.

### Polyacrylamide gel electrophoresis (PAGE)

As an alternative to agarose gel electrophoresis, polyacrylamide gels can be used for the analytical or preparative separation of small, double-stranded DNA fragments. This method is applicable to DNA fragments from 10 to 1000 bp. The resolution and capacity of polyacrylamide gels is higher than that of agarose gels. However, agarose gels are much easier to pour and run, and in the vast majority of cases deliver acceptable resolution. Protocols for PAGE of DNA can be found in standard molecular biology texts (1, 2).

## Quantification of DNA

Reliable measurement of DNA concentration is important for many applications in molecular biology. Plasmid DNA quantification is generally performed by spectrophotometric measurement of the absorption at 260 nm, or by agarose gel analysis. In this section, we examine some critical factors for quantification, such as the effect of solvents, phenol, and RNA contamination on absorption.

### DNA quantification by spectrophotometry

Plasmid DNA concentration can be determined by measuring the absorbance at 260 nm ( $A_{260}$ ) in a spectrophotometer using a quartz cuvette. For reliable DNA quantification,  $A_{260}$  readings should lie between 0.1 and 1.0. An absorbance of 1 unit at 260 nm corresponds to 50  $\mu$ g plasmid DNA per ml ( $A_{260} = 1 \Rightarrow 50 \mu\text{g/ml}$ ).<sup>\*</sup> This relationship is only valid for measurements made at neutral pH, therefore, samples should be diluted in a low-salt buffer with neutral pH (e.g., Tris·Cl, pH 7.0). An example of the calculation involved in nucleic acid quantification when using a spectrophotometer is provided in "Spectrophotometric Measurement of Nucleic Acid Concentration", page 91.

When working with small amounts of DNA, such as purified PCR products or DNA fragments extracted from agarose gels, quantification via agarose gel analysis may be more effective (see "DNA quantification by agarose gel analysis", page 17).

**Tip** If you will use more than one quartz cuvette to measure multiple samples, the cuvettes must be matched.

<sup>\*</sup> Based on a standard 1 cm path length.